A NEW TOOL TO DETECT STRESS AT THE METABOLIC LEVEL IN AQUATIC INSECTS

Presented by:
Scott Johnson & Jesus Reyes

California Aquatic Bioassessment Workshop
October 30th, 2013

Acknowledgements

- Rich Gossett, Dr. Kevin Kelly – IIRMES, CSULB
- Jesus Reyes - Pacific Coast Environmental Conservancy
- Wendy Willis – Aquatic Bioassay & Consulting Laboratories, Inc.
- Dr. Kristy Morris – Council for Watershed Health

San Gabriel River Monitoring Program Stakeholder Group

SANITATION DISTRICTS OF LOS ANGELES COUNTY

Council for Watershed Health

LOS ANGELES COUNTY FLOOD CONTROL DISTRICT

www.ocwatersheds.com
OC Watersheds Program
County of Orange

SCCWRP
Established 1969

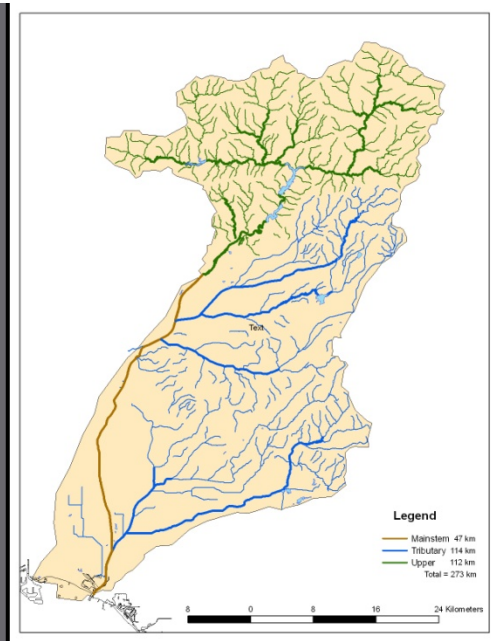
CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS

Outline

- Background on the San Gabriel River Regional Monitoring Program (2005 to present)
- Stressor Identification
  - MLOE Approach
- Proteomics
  - Background
  - Study Design & Methodology
  - SGRRMP Special Study Results
- Going Forward

Upper Watershed

Lower Watershed

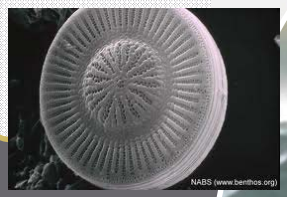


Main Stem

Estuary

Stream Condition Monitoring Indicators

- Benthic Macroinvertebrates
- Attached Algae



- General Constituents
- Metals
- Nutrients
- Organics

Water Chemistry

Stream Condition ?

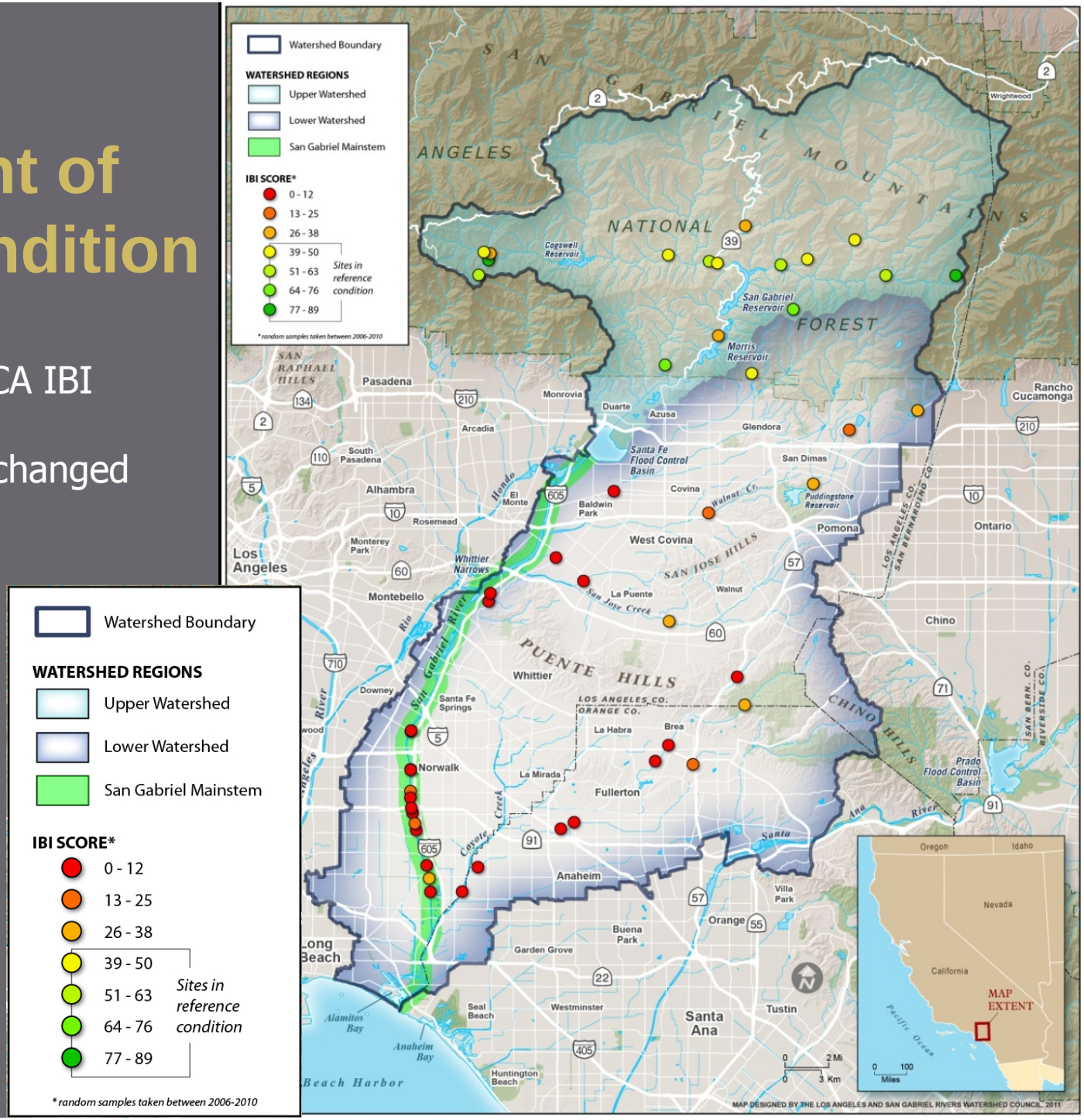
Physical Habitat

Toxicity

Surveys: SWAMP and CADF&G
California Rapid Assessment Method (CRAM)

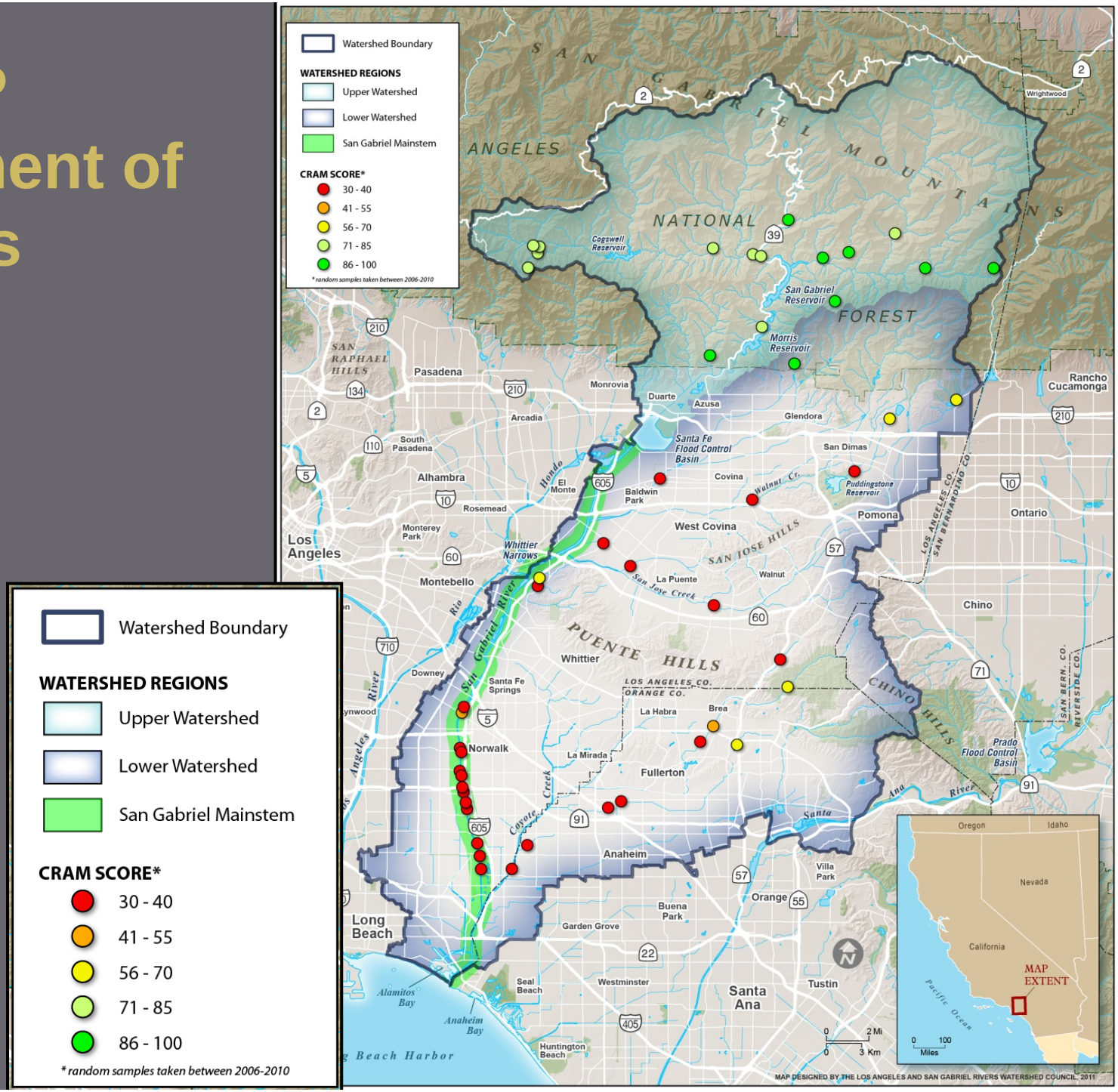
SGRRMP Assessment of Stream Condition

- Multimetric So CA IBI
- Scores ≤ 39 = changed



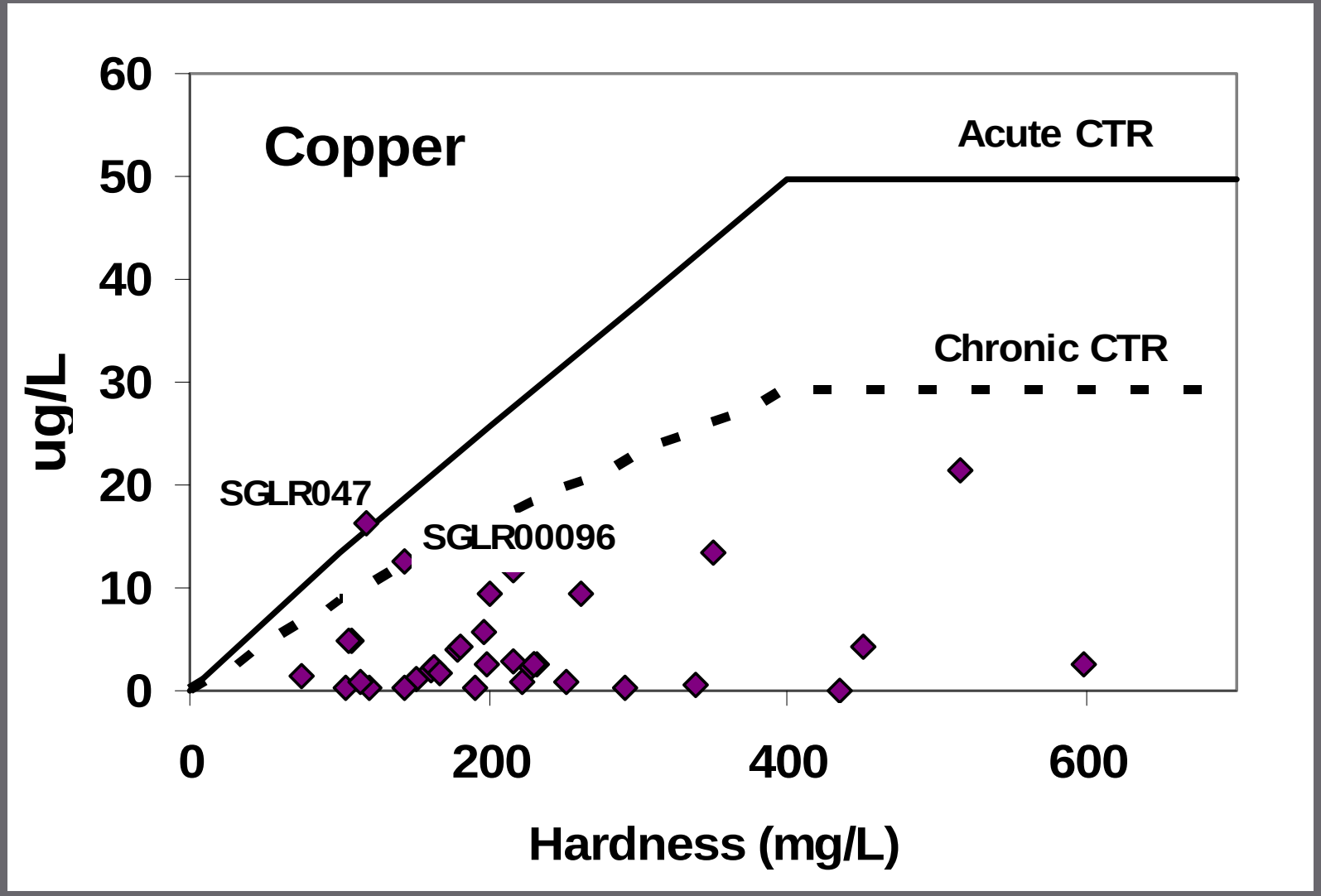
SGRRMP Assessment of Stressors

- CRAM



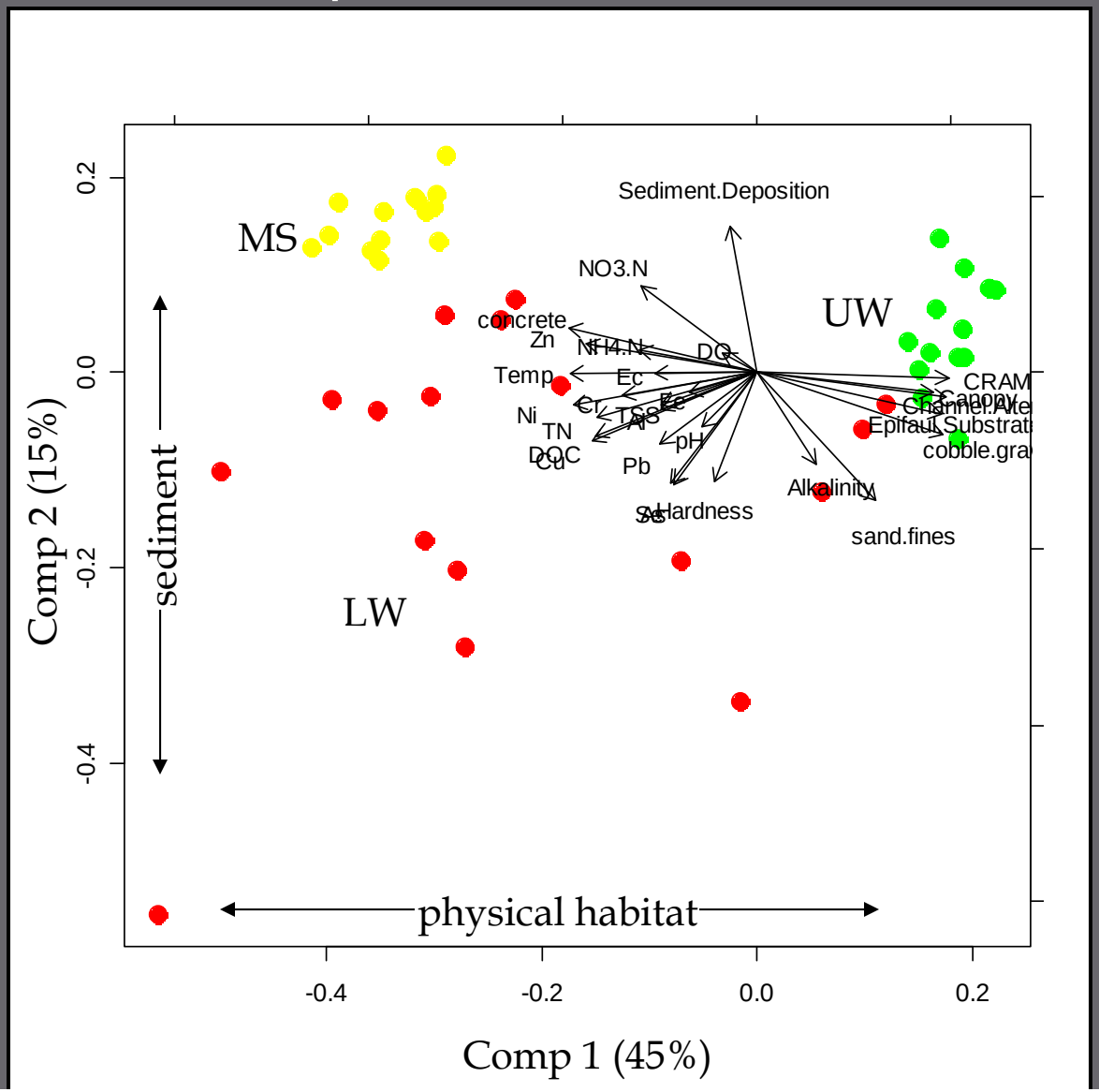
SGRRMP Assessment of Stressors

- CA Toxics Rule (CTR): hardness adjusted dissolved metals



SGRRMP Assessment of Stressors

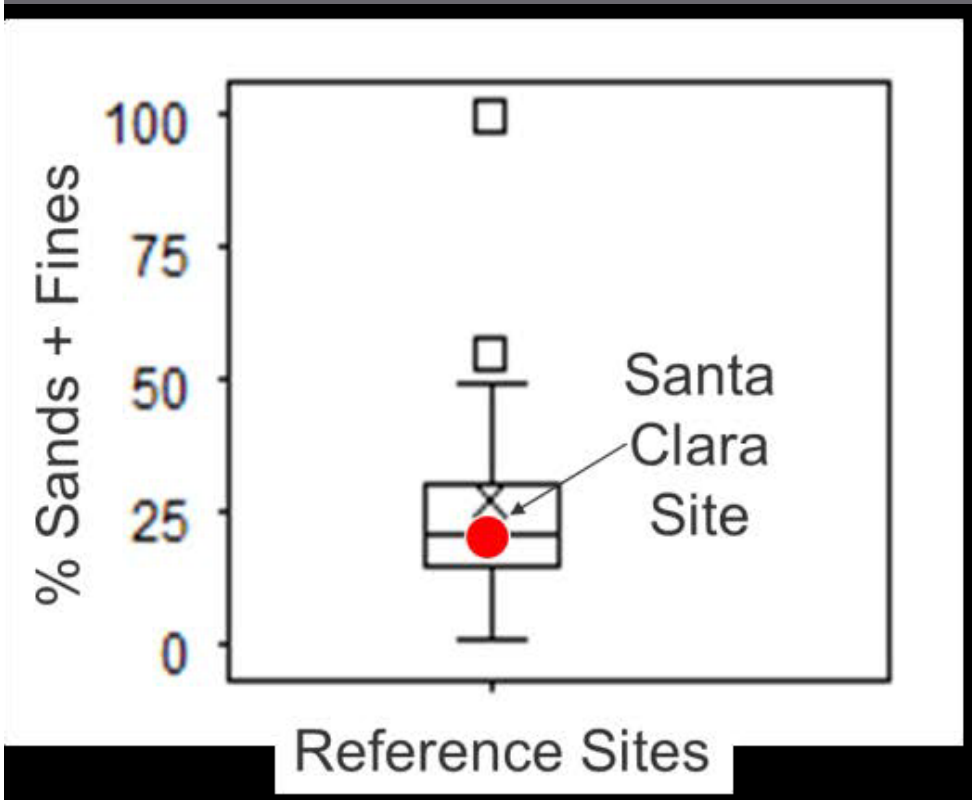
- PCA Biplot



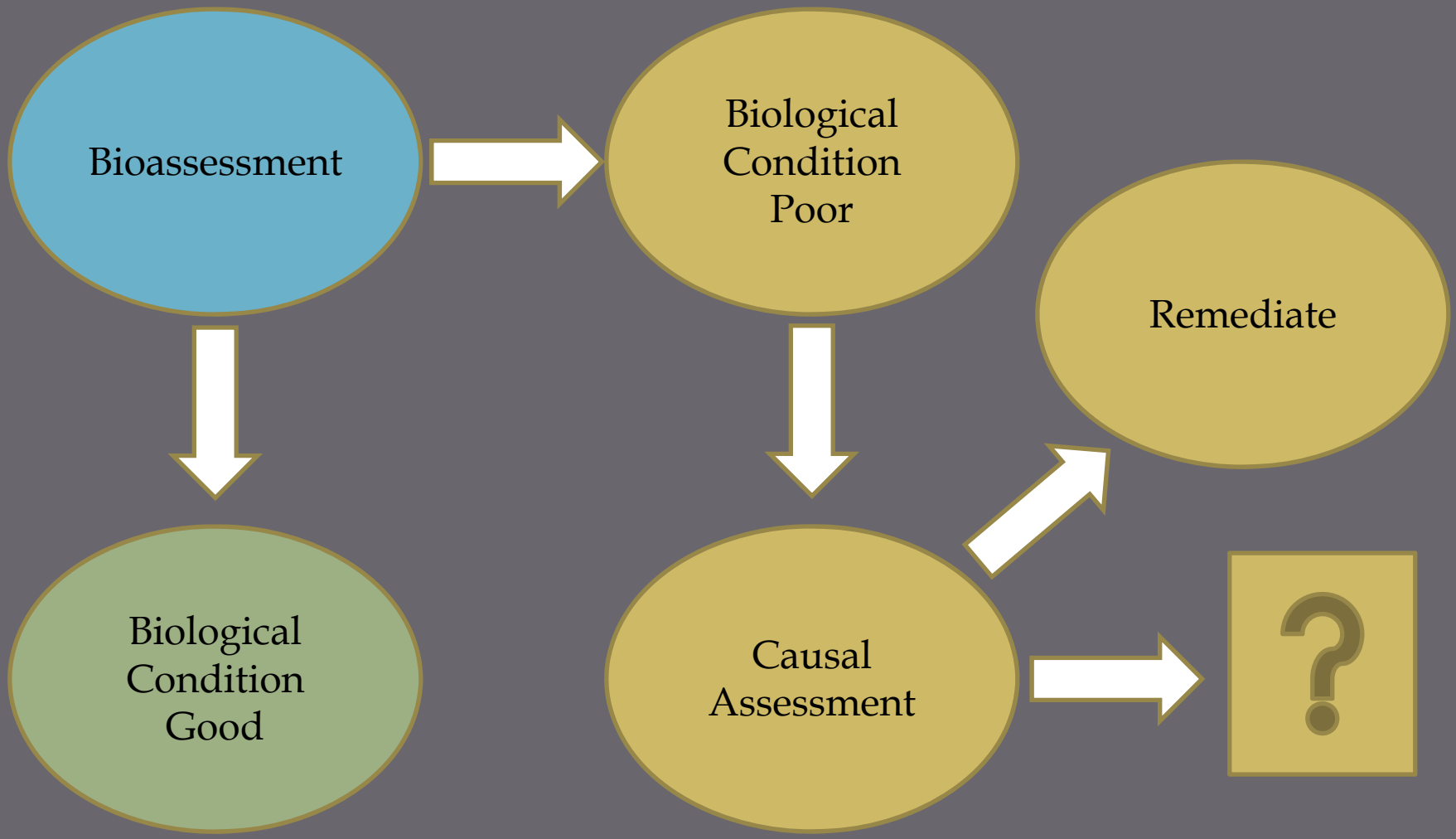
- Comp 1 (45%)
  - CRAM
  - epifaunal substrate
  - % cobble
  - % canopy
- Comp 2 (15%)
  - sediment deposition
  - sands
  - fines
- Comp 3 (10%) ions
  - alkalinity
  - hardness
  - pH
  - Ec

Causal Assessment

- Build associations between site condition and reference



How certain are we?



What if the bugs could tell us what's stressin' em?

Animal Phenotypic Response Measurements

- Measure using biomarkers as methodology in understanding environmental (water quality) effects
- **Endocrine Responses** [environmental endocrinology]
- **Tissue Expression Responses** [proteomics, genomics]

Animal Phenotypic Response Measurements

- Measure using biomarkers as methodology in understanding environmental (water quality) effects
- **Endocrine Responses** [environmental endocrinology]
- **Tissue Expression Responses** [**proteomics,** genomics]

Proteomics

Mapping and quantification of an organisms entire set of cellular proteins

→Phenotypic responses

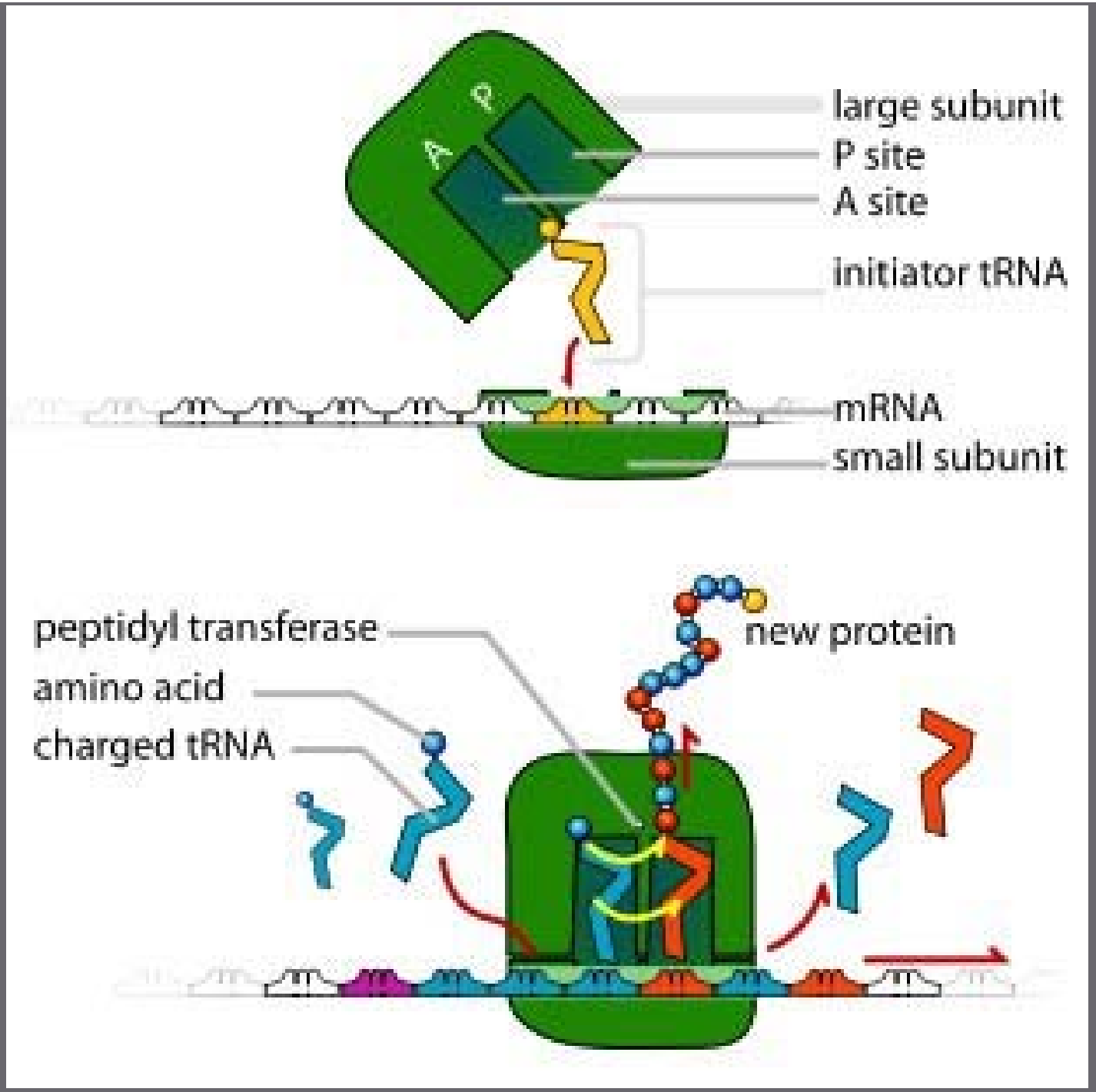
reproductive, growth & development, toxicological, metabolic

→Environmental factors

distinct biomarkers *(protein, endocrine, other)* reflect distinct environmental factors *(chemical, physical, other)*

→Development of each new biomarker adds to subsequent diagnostic power

→Prospects for detecting effects even when identity of environmental contaminant is not yet known


P
A
large subunit
P site
A site
initiator tRNA
mRNA
small subunit
peptidyl transferase
amino acid
charged tRNA
new protein

Proteomics

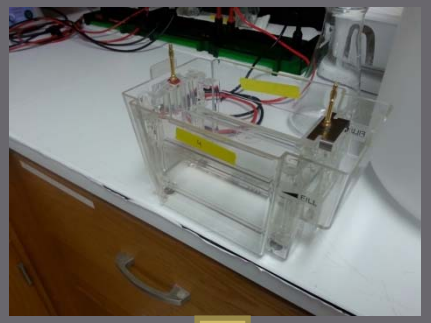
- Volume of protein produced differs in response to amount and duration of stress
- Protein maps are specific to each species and are reproducible
- Protein maps can be overlaid to assess protein expression among locations

Lab Methods

Sonicate & prep

Isoelectric focusing (IEF) on 2-D strip

Separate by molecular weight on 2-D gel



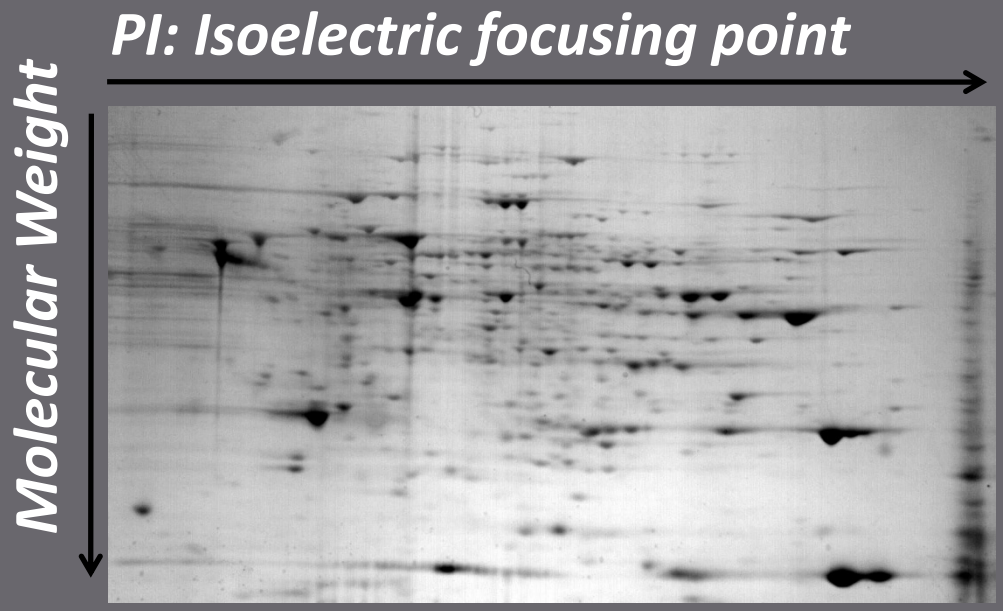
Dye proteins on gel

Pick proteins for ID

ID proteins using mass spec, local and external databases

IEF: isoelectric focusing

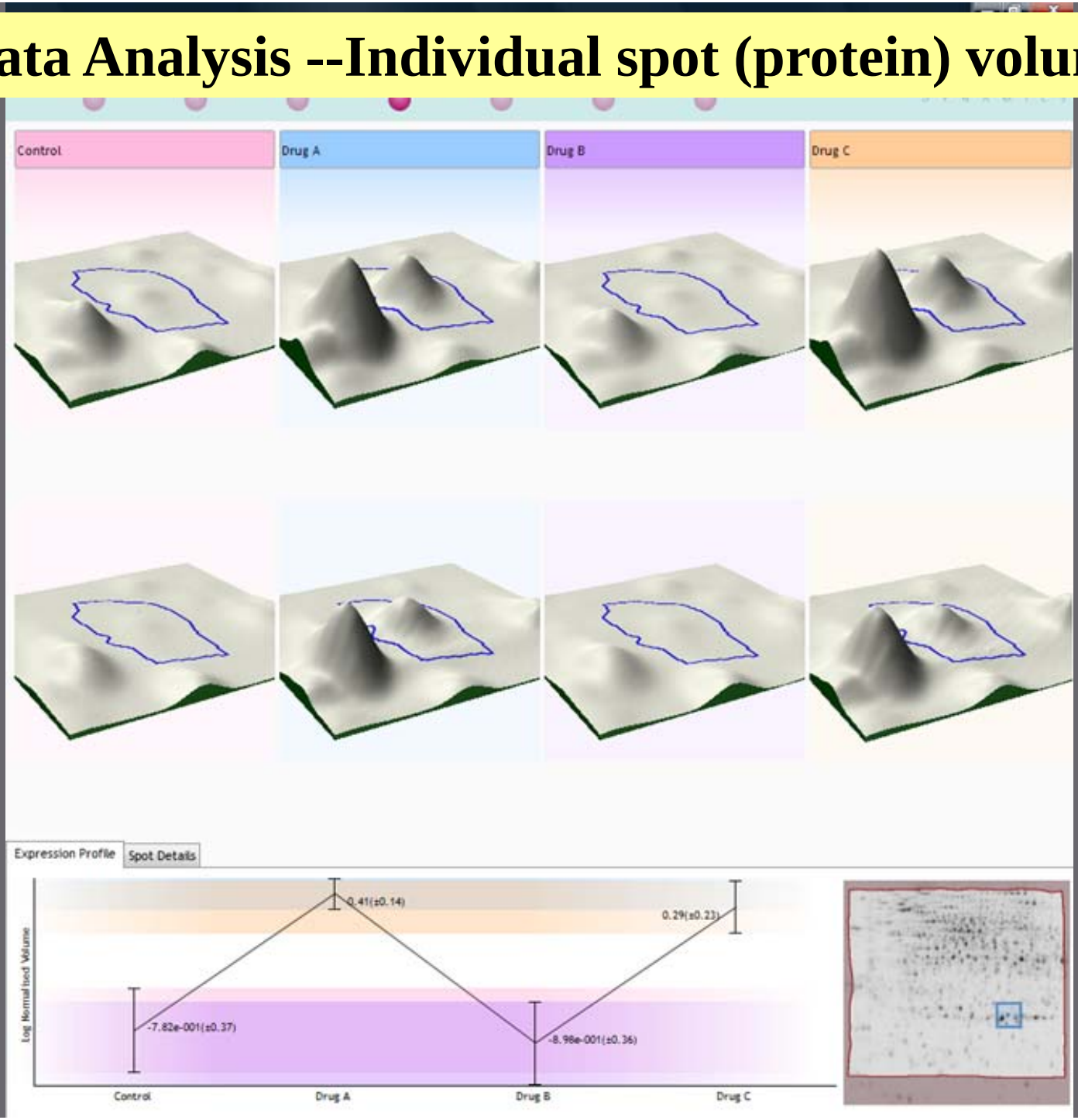
- Samples run on a 2-D gel which separates proteins by PI horizontally and mass vertically



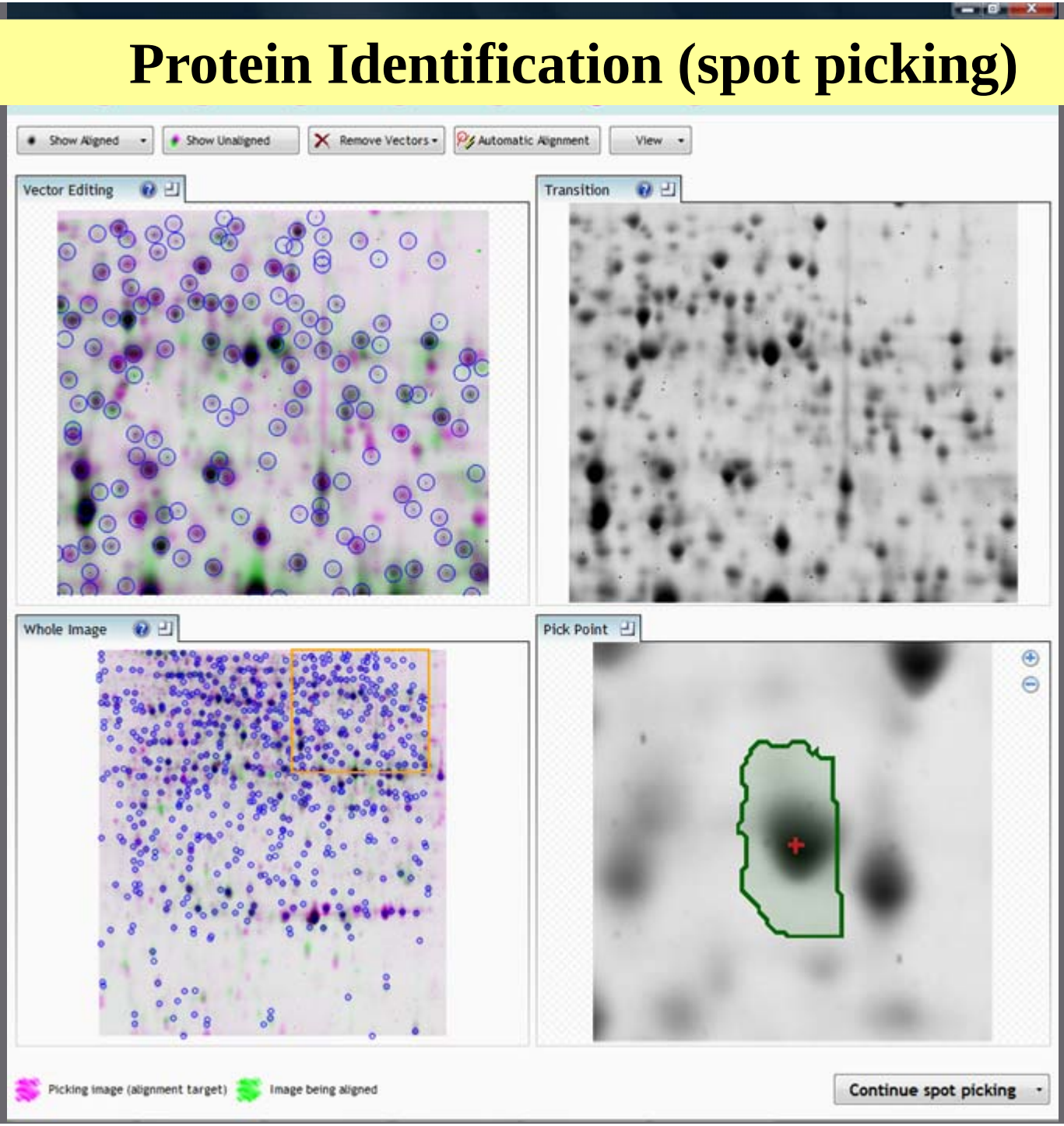
English sole

- This results in a unique protein profile for each organisms from each environment (unique fingerprint)

Data Analysis --Individual spot (protein) volume



Protein Identification (spot picking)

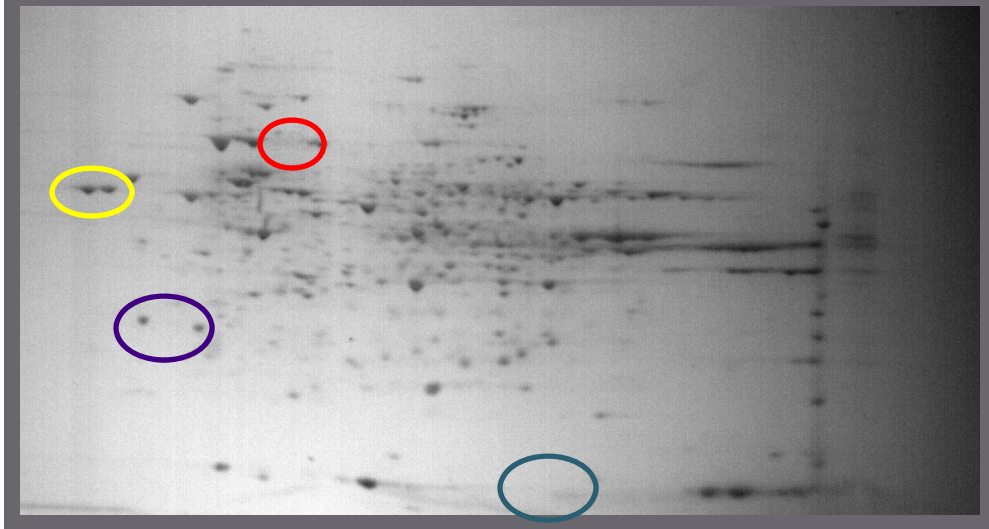


Colorado Lagoon Long Beach, CA

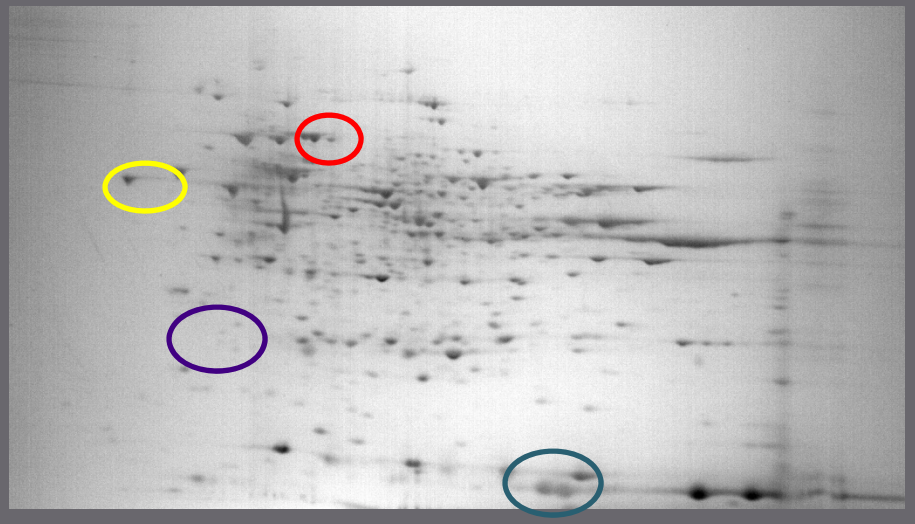
http://www.flickr.com/photos/crankyflier/3561009460/

Pacific Coast Environmental Conservancy

Comparison of Multiple Biomarkers (Proteomics)



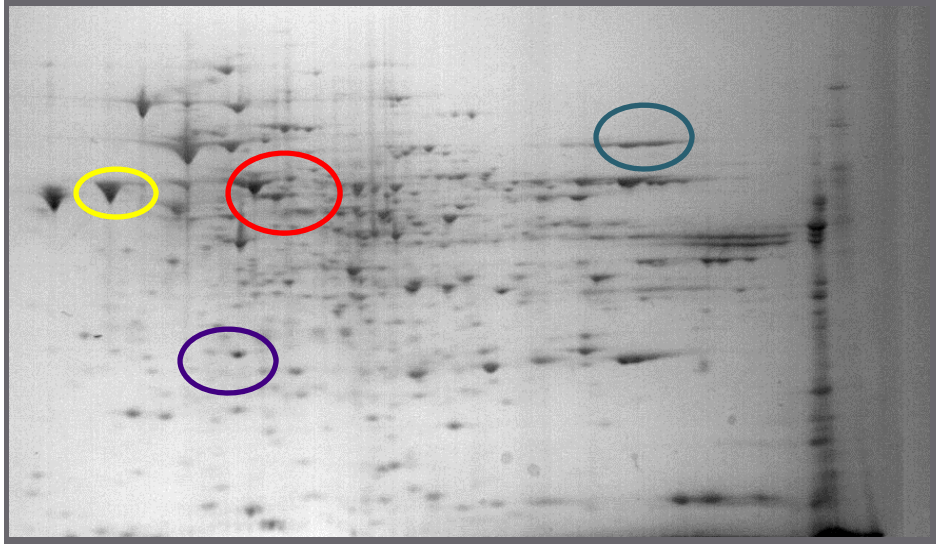
Colorado lagoon

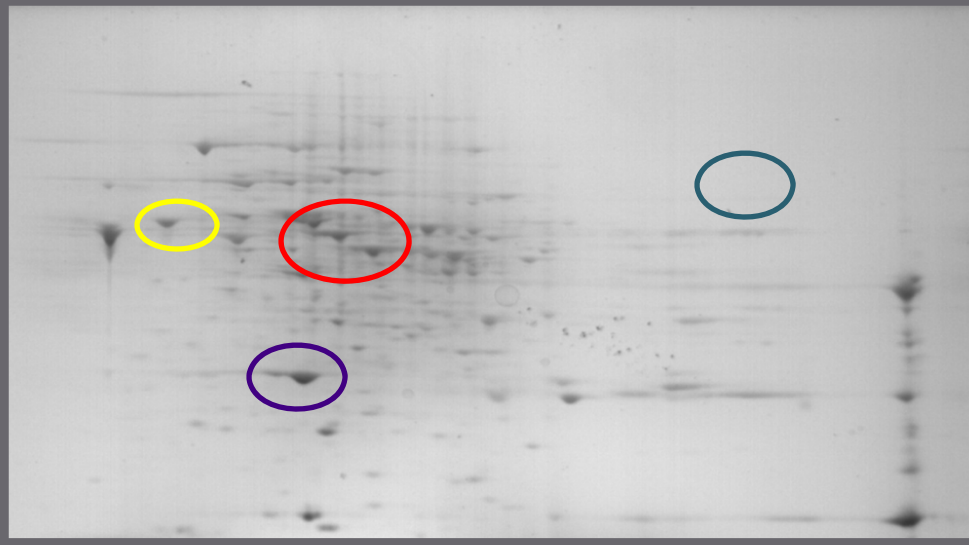


Redwood city

Pacific Coast Environmental Conservancy

Comparison of Multiple Biomarkers (Proteomics)





Colorado lagoon

Redwood city

Pacific Coast Environmental Conservancy

Special Study Question(s)

- Can proteomics be used to map protein expression in aquatic insects?
  - Can differences in protein expression be detected in the same species collected at reference and non-reference stream reaches?

Method Development

- Two species common in both the upper and lower San Gabriel River watersheds.

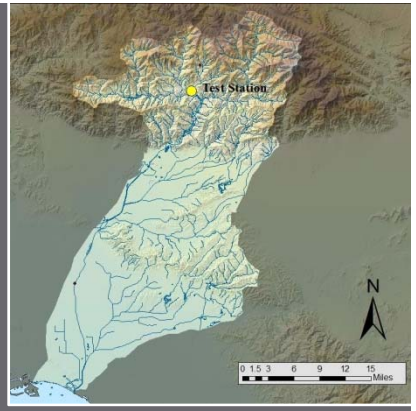
Hydropsyche sp.

DFG-ABL

Baetis adonis

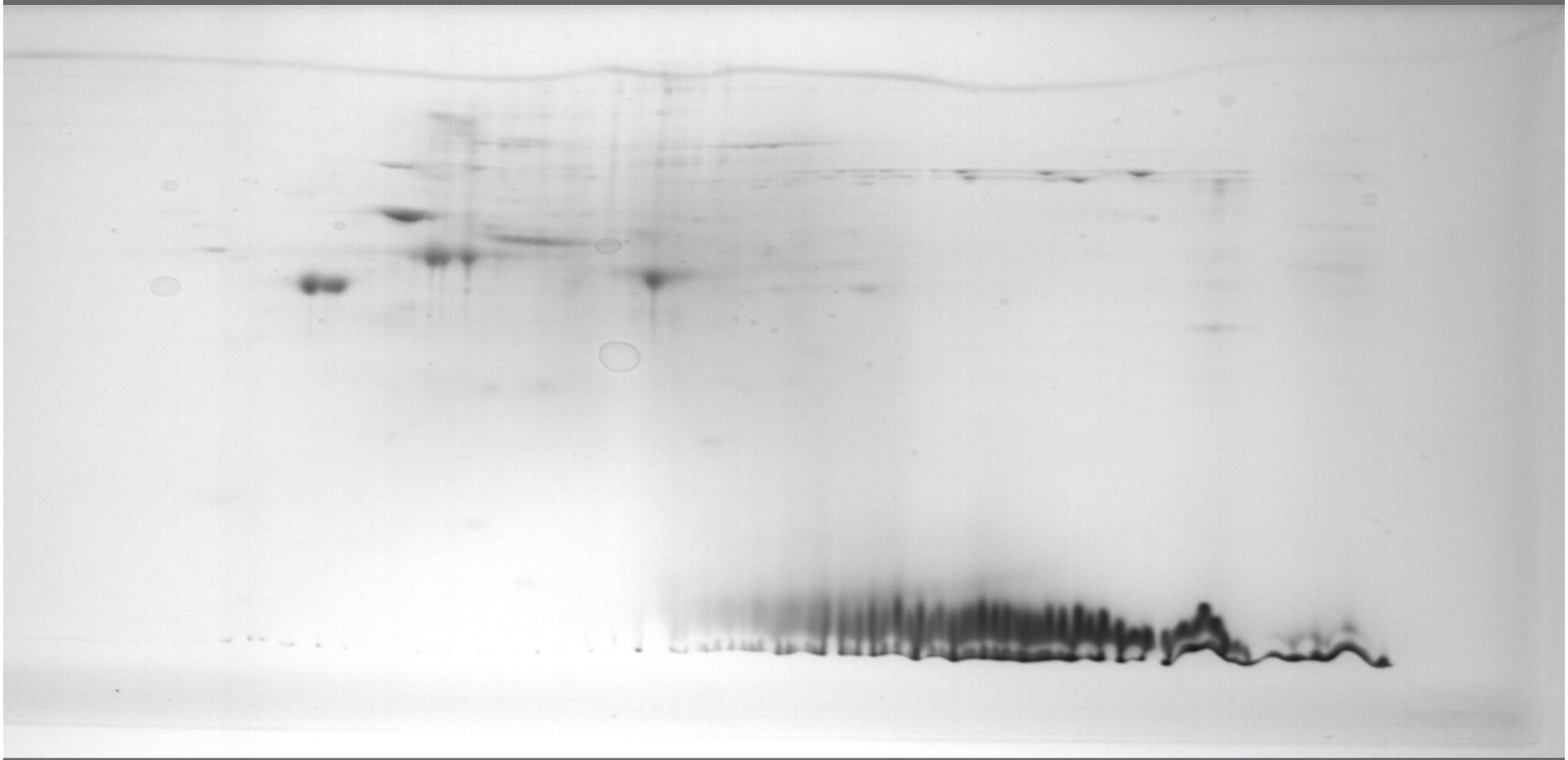
DFG-ABL

Method Development

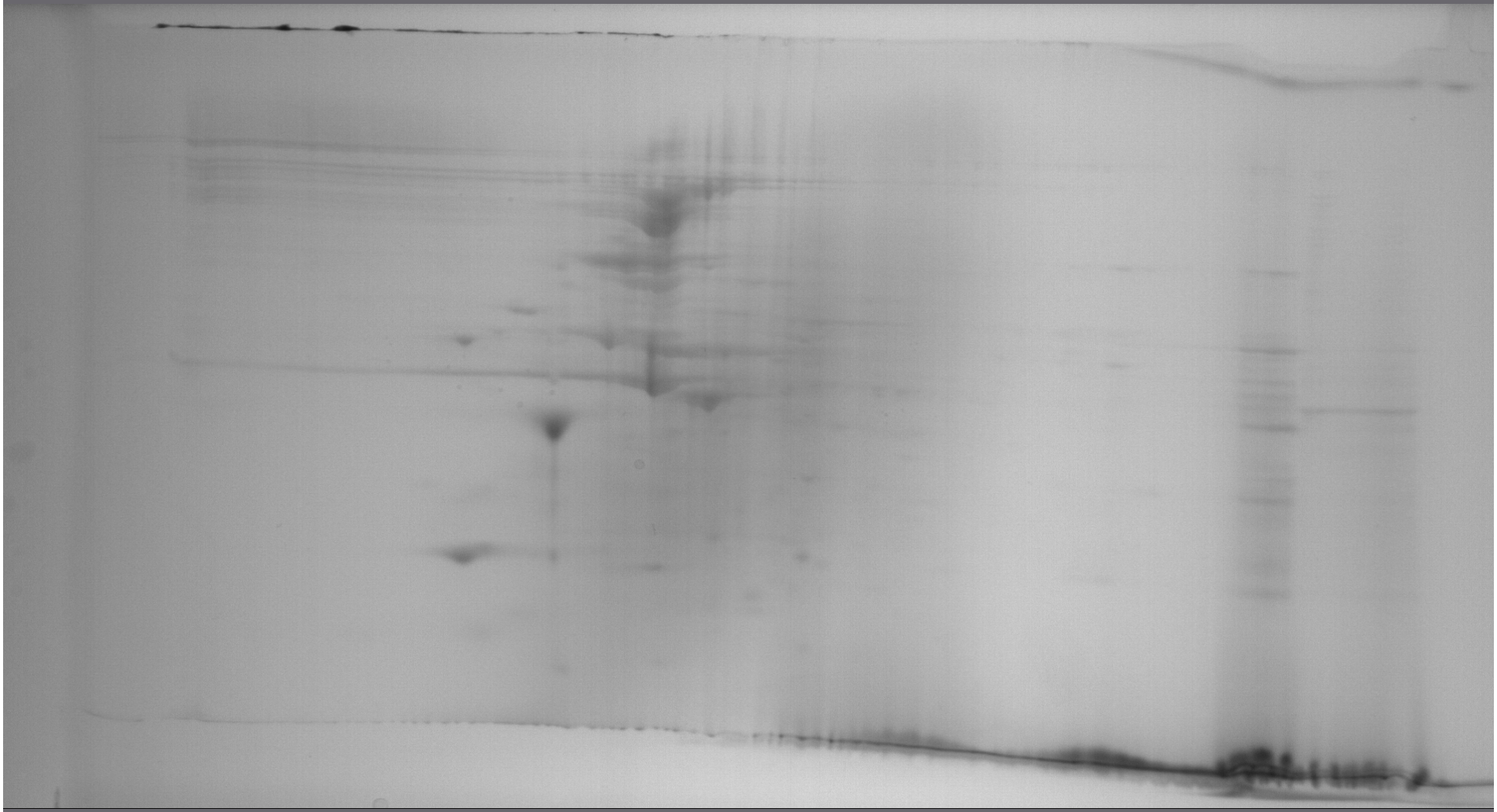


- The initial attempts at protein mapping were unsuccessful.
  - Due to using older specimens or ETOH
- Two separate samples were collected from the West Fork SGR
  - One sample preserved with 95% ETOH on ice
  - One sample held in water on ice
- The sample stored in cold sample water showed strong signals

Results : *Hydropsyche sp.*



Results: *Baetis adonis*



Protein Identification

TABLE I -- PROTEIN IDENTIFICATION RESULTS

Proteins with Scores ≥100				
plug # / position	Protein Name		protein score	protein ci
N3	Arginine kinase	1	110	100
N4	Arginine kinase	1	140	100
N6	Tropomyosin-2	2	228	100
N7	Tropomyosin-2	2	372	100
N21	ATP synthase subunit beta, mitochondrial	3	451	100
N22	ATP synthase subunit beta, mitochondrial	3	350	100
O1	Arginine kinase	1	104	99.998
O3	Trophomyosin-2	2	126	100
O11	Heat shock 70 kDa protein cognate 3	4	164	100

Protein Identification

Stress Response/Detox

- *HSP70*
- *Metallothionein-protein type 2*
- *Conotoxin*
- *Hepcidin*
- *Glutathione S-transferase*

Metabolism

- *Arginine kinase*
- *DNA directed-RNA polymerase*

Structural & Transport

- *Tropomyosin-2*
- *Troponin*

Cell Signaling

- *ATPase synthase*
- *Apoptosis regulator*

Discussion

- Can proteomics be used to map protein expression in aquatic insects?
- Preservation and storage techniques were optimized
- Provided the beginnings of species specific biomarker database for aquatic insects

Discussion

- Can proteomics be used to map protein expression in aquatic insects? Yes !!!!
- Preservation and storage techniques were optimized
- Provided the beginnings of species specific biomarker database for aquatic insects

Discussion

- Can proteomics be used to map protein expression in aquatic insects? Yes !!!!
- Preservation and storage techniques were optimized
- Provided the beginnings of species specific biomarker database for aquatic insects
  - More work needs to be done to create comprehensive protein profiles

Discussion

- Lessons Learned
  - Hold times are short for organisms since they are collected live and held on ice.
  - Finding species resident in both reference and non-reference locations not as easy as it sounds.
  - What are the effects of collection and preservation on gene expression?

Future Studies

- Can differences in protein expression be detected in the same species collected at reference and non-reference stream reaches?
- Build protein database for several species

Questions????

Scott Johnson
Aquatic Bioassay & Consulting Laboratories, Inc.
scott@aquabio.org

Jesus Reyes
Pacific Coast Environmental Conservancy
reyes@pceconservancy.org